STANFORD UNIVERSITY

Department of Psychology

Stanford, California

May 28, 1965

TO: Office of Grants and Research Contracts
Attention: Code SC, National Aeronautics
and Space Administration
Washington, D.C. 20546

RE: Semi-Annual Progress Report
("Effects of Antimetabolites on Learning
and on Brain Metabolism")
NASA Research Grant NGR-05-020-081

The first six months of the period covered by this grant have been devoted to establishing necessary laboratory facilities and working out techniques to be used in the projected research on antimetabolite effects on brain metabolism and on learning.

The experiments have two aspects - biochemical and behavioral. The biochemical work, which is being carried out in collaboration with Dr. Jacob Shapira at the Ames Laboratory in Mtn. View, has involved analyses of selected brain tissues to determine rates of RNA and protein turnover in normal and experimentally-drugged rats. The RNA determination has been made by a modification of techniques successfully used by Dr. Shapira with tissue culture. Our initial work has been largely devoted to demonstrating that these techniques yield sufficiently accurate data for various regions of rat brain under the conditions of our experiments. The primary problems were straightforward ones of arriving at the proper kinds and amounts of radioactive precursors of RNA and protein and the best vehicle and route of administration. After a number of preliminary experiments, we have established a standard injection solution consisting of 80 microcurie C^{14} leucine, 200 microcurie H^3 cytidine, 20 mg cytidine sulfate, 8 mg OL leucine in Hanks basic salt solution. This solution is injected by direct heart puncture into unanesthetized rats. One hour later, the animals are sacrificed and the brains, hearts and livers quickly frozen in alcohol and dry ice. At a later time, the brains are dissected in the frozen state and samples from several parts of the cortex, subcortical areas, cerebellum, brain stem, heart and liver are taken. These samples are separated into RNA, protein, and low molecular weight fractions for counting and ultra-violet spectrophotometry. Initially, we encountered some difficulty in getting tagged precursors for RNA into brain tissue. However, a change from uridine to cytidine and the addition of a fairly large amount of non-labeled cytidine to the injection solution has largely overcome this difficulty. While we have no exact quantitative knowledge as yet, we estimate that our present

technique probably allows us to detect changes in RNA synthesis of approximately 10 to 20 percent, and probably somewhat better for protein. This is for single animals. Naturally for several animals our statistical accuracy is considerably better.

A somewhat more difficult problem has been the choice and administration of anti-metabolites. To date, we have worked with only TCAP and actinomycin D. In neither case have we effected a large change in brain metabolism. In the case of TCAP we have used dosages and animals (rats) as nearly the same as possible to those used by other investigators who have presumably obtained results mediated by alterations in brain RNA synthesis. We have not detected any changes in brain RNA resulting from cardiac injections of TCAP. These experiments are continuing, however, and the present negative results must be viewed as very tentative and preliminary.

We have tried a few experiments with actinomycin D. We have not detected any effect on brain RNA synthesis with this drug at the dosages we have used. The dose, given IP to young rats, has been 1.25 mg/Kg. However, this dose is far below that used by other investigators (Jarvick and Barondes, 1964) and by a different route - IP instead of intracranially, and in a different animal - rats instead of mice. We are just now beginning to do experiments using mice, and we plan to use larger doses and intracranial injections, as these other investigators have. The use of mice will also affect an economy with respect to the amount of labeled biochemicals which we will need to use.

The other aspect of our work is the effects of anti-metabolite drugs on learning. Along this line, we have been working out standard learning assay techniques. We have settled on the use of a passive avoidance task and a food-motivated Y maze. Our procedure is to alternate one trial in each apparatus every half-hour during an eight-hour period following the administration of a drug, such as actinomycin D. This allows us to study two very different kinds of behaviors, both being learned with fairly widely spaced trials so that drug effect on memory consolidation are involved. In experiments done during the period under report here, various preliminary forms of this technique were employed. A series of experiments was done using the drug TCAP and studying passive avoidance in an "elevator box," and maze behavior motivated both by shock escape, and food approach. To date, no consistent results with immediate interpretation have been obtained with TCAP, although some suggestive data have been obtained. The most intriguing suggestion, however, is that TCAP may have little or no effect on learning in the tasks which we have studied, as contrasted to positive results previously reported by others. Considerably more work is needed before we can draw any firm conclusions, however. Further experiments on TCAP with rats are planned for this summer.

Actinomycin has been used in experiments with rats, and the passive avoidance response. At the dosages and with the routes of administration which we used, we found a fairly clear lack of effect of this drug on learning. However, as previously noted, we found a corresponding lack of its effect on brain RNA synthesis. This despite the fact that the dosages were uniformly fatal within a period of 24 hours.

In summary, our progress to date has been reasonably in line with our plans and expectations. We have our laboratory equipped and set up for the experiments which were projected in the proposal, and we have progressed satisfactorily in working out the details of techniques and procedures. Our initial preliminary experiments have not produced dramatic findings, but they have produced some information, leads, and firmer control over our techniques. Our original plan was to be ready for a major series of experiments during the summer of 1965. This involves having done the necessary pilot studies and having established the methods and procedures to be used, so that a number of studies can be carried on simultaneously, and with fairly large numbers of animals. It appears that by the middle of June we should be ready to do just that.

No publications have been submitted as a result of this research as yet. One Honors thesis at Stanford University has resulted from the project, but its experiments will be repeated before they are reported.

Thomas K. Landauer
Principal Investigator

TKL:sm